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ADENOVIRAL MEDIATED ß-GALACTOSIDASE (*lacZ*) REPORTER GENE EXPRESSION IN THE MOUSE RETINA

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Pupose: In order to develop a treatment for recessive retinitis pigmentosa the ultimate objective is to introduce a normal copy of the abnormal gene into photoreceptors. The post-mitotic status of photoreceptor cells make them unsuitable targets for existing retroviral vectors. One of the strategies that we have been exploiting is adenoviral (AV) mediated gene transfer. Here we report successful transduction of ocular tissues including neuroretina and retinal pigment epithelium (RPE) with a lacZ reporter gene using an adenoviral vector.
Methods: The recombinant adenovirus, pXCXRBb, contains an *E.Coli* ß-galactosidase gene driven by a Rous Sarcoma virus promotor and with SV40 polyadenylation signals. Approximately 0.4µl of viral suspension containing pXCXRBb at 10^9 pfu/ml was injected with a 1.5 cm, 34-gauge hypodermic needle into the vitreous and into the subretinal space of HsdOla:MF1 mice. Control MF1 and BALB/cOlaHsd eyes were injected with an equivalent volume of PBS. Animals were perfused 4,7 and 14 days postinjection with 2% paraformaldehyde and 0.05 % glutaraldehyde in 0.1M phosphate buffer. The eye cups were incubated at room temperature with X-gal overnight to detect *lacZ* activity (blue staining) in transduced cells. The tissue was paraffin embedded and sectioned at 5-10 µm thickness. Sections were counterstained with nuclear fast red and examined by light microscopy.
Results and Conclusion: Fine blue staining of the RPE and of iris- and ciliary body epithelium was observed in BALB/c controls indicating endogenous ß-gal activity which makes evaluation of transduction after intravitreal injections difficult in these mice. This was not seen in control MF1 mice which we therefore used in our subsequent experiments. Distinct ß-gal staining of RPE cells, photoreceptor cells and optic nerve glia cells was only detected in injected eyes and abundant near the injection site. Over time, the percentage of lacZ-positive cells decreased and is highest during the first week post injection. Whether this is due to an immune response against cell transduced by the AV vector remains to be determined by ongoing experiments. Our results to date suggest that the current generation of replication deficient adenoviral vectors may be a tool of limited use for gene transfer into the retina. However, this work will serve as a baseline for further development of gene therapy of RP for which application of a non pathogenic, integrating DNA vector such as adeno associated virus with a photoreceptor cell specific promotor may have higher potential.

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RETINAL DEGENERATION IN THE *rds* MOUSE IS NOT *p53* DEPENDANT.

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Purpose: Mutations in the ß-PDE, peripherin-*rds* and rhodopsin gene cause retinal degeneration in mice as well as humans. The fact that apoptosis is the final pathway in each of the different mouse phenotypes suggests that a common mechanism is involved. The tumour suppressor gene *p53* is involved in various apoptotic pathways in the mouse eye including apoptosis in the developing lens and apoptosis due to the viral proteins, human papillomavirus -16 E7 and adenovirus E1A. The aim of this experiment is to elucidate the role of *p53* in the apoptosis seen in *rds* mice. We therefore generated animals that were deficient in the *p53* gene and homozygous for the *rds* mutation.

Methods: Mice homozygous for a targeted disruption (10kb deletion) of the p53 gene (obtained from A.R. Clarke, Dept. of Pathology, University of Edinburgh) were crossed with mice carrying the rds mutation (obtained from D. Bok, Jules Stein Eye Institute, Los Angeles). F1s -all double heterozygotes (p+/r+)- were intercrossed and the F2 offspring were genotyped by PCR from tail DNA. Mice were sacrificed at 2 months and their eyes examined histologically. Semi-thin sections were taken at the optic nervehead level and subsequent nuclear counts and measurement of the thickness of the different retinal layers were used to asses morphological differences.

Results and Conclusion: At 2 months the degeneration in an *rds* mouse is about 50% complete with the photoreceptor cell layer being reduced to half its thickness. Analysis of mice at 2 months carrying the pp/rr genotype showed similar degeneration to that of p+/rr and ++/rr siblings and the original *rds* parent. Our results to date suggest that retinal degeneration in the rds mouse occurs via an apoptotic pathway that is p53 independent. This is to be confirmed by agarose gel electrophoresis to detect apoptosis-specific internucleosomal DNA fragmentation.

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BIETTI'S CRYSTALLINE RETINOPATHY

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Purpose : Bietti's crystalline dystrophy is a retinal degeneration beginning in the third decade of the life characterized, by tiny yellow crystals in the posterior pole and the superficial paralimbic cornea. We report a case of bietti's crystalline dystrophy without marginal corneal dystrophy in a 44 years old man. Methods : a complete ophthalmological examination, including color vision test. Goldmann perimetric visual fields, fluorescein angiography, Ganzfeld electroretinogramm (E.R.G) electro-oculogram (E.O.G), evoked visual potentials (E.V.P) was performed. Results : In this case a history of night blindness and visual impairment have been present for a few years. Recently, his visual acuity is 2/10 in the right eye and less than 1/40 in the left eye, the visual fields show a bilateral central scotoma. No corneal abnomalities were found. The fundus shows tiny yellow crystals and retinal pigment epithelium defects localized in a circular area at the posterior pole, this lesion is accompanied by numerous small areas of retinal degeneration at the mid peripheral retina. The E.R.G. was normal, the E.O.G was profoundly abnormal, the E.V.P were perturbated. The patient had arterial hypertension and hypercholesterolemia. Plasma and urine levels of amino-acids were normal. Conclusion : Bietti's crystalline dystrophy is a rare form of retinal degeneration. Usually associated with a marginal corneal dystrophy. The authors present a localized type of this discase with typical history, fundus appearance and angiographic features, they also discuss differential diagnosis.

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PHENOTYPICAL AND GENOTYPICAL CLASSIFICATION OF AUTOSOMAL RECESSIVE RP

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Purpose
To perform clinical and DNA research in a large pedigree with autosomal recessive retinitis pigmentosa (arRP).
Methods
A large pedigree from a genetic isolate in the Netherlands, with 45 affected persons with arRP, was examined ophthalmologically. Blood samples were collected for DNA analyses.
Results
Initially, the arRP pedigree suggested the occurrence of one type of RP with one single gene. Extensive clinical examination revealed different phenotypes: 22 patients showed RP with preserved para-arteriolar retinal pigment epithelium (PPRPE), 5 showed retinitis punctata albescens, 4 RP with geographic atrophy of the macula, 2 pericentral retinal dystrophy, and 12 had Usher's syndrome (type II). DNA linkage analysis and genetic homogeneity analysis resulted in the assignment of one gene for RP with PPRPE to chromosome 1q31-32.1, and the presumption that at least one more gene is likely to segregate in this pedigree.
Conclusion
The phenotype and genotype of autosomal recessive RP is, even within a single large family, more heterogeneous than previously anticipated in the literature.